The Ivomec SR Bolus for Control of *Boophilus annulatus* (Acari: Ixodidae) on Cattle in South Texas

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ABSTRACT When Hereford heifers infested with *Boophilus annulatus* (Say) were treated with a single Ivomec SR Bolus, the concentration of ivermectin in the serum of the treated cattle reached a maximum of 8.8 ± 0.9 ppb at 2 wk posttreatment. The single bolus treatment resulted in 84.4% control of standard engorging *B. annulatus* females on treated cattle over the 20-wk trial. Although fewer engorged ticks were collected from the sentinel heifers exposed in the treated pasture than those in the control pasture at weeks 4, 10, and 16 posttreatment, none of the differences was statistically significant. Each exposure of sentinel cattle found free-living ticks in both the treated and control pastures, indicating the infestation was not eliminated by the treatment. When the trial was repeated using two Ivomec SR Boluses/heifer, the concentration of ivermectin in the serum of the treated cattle reached a maximum level of 31.2 ± 3.9 ppb at week 13 posttreatment. The use of two boluses/heifer resulted in 99.6% control of standard engorging *B. annulatus* females over the 20-wk trial. No ticks were found on sentinels placed in the treated pasture after week 9 posttreatment, an indication that the treatment had eliminated the free-living population in the treated pasture. From these studies, we conclude that a single Ivomec SR Bolus is incapable of sufficient control of *B. annulatus* to meet the rigid requirements of the Cattle Fever Tick Eradication Program in South Texas. Although two boluses per animal did eliminate the ticks from treated heifers and the pasture they were in, the treatment would not be sufficiently efficacious for mature cattle (>400 kg) for it to be useful in the program.

KEY WORDS *Boophilus annulatus*, ivermectin, sustained release bolus

BECAUSE OF THE United States *Boophilus* Eradication Program, *Boophilus* spp. ticks and *babesiosis*, the disease transmitted by these ticks, have been eradicated throughout the country with the exception of eight counties that lie along the Texas-Mexico border. A permanent quarantine, maintained as part of the Cattle Fever Tick Eradication Program, is enforced by the U.S. Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS), Veterinary Services both to prevent reintroduction of the ticks into the U.S. and to eradicate infestations when ticks are found here (Graham and Hourrigan 1977). Standard procedures for the eradication of a new infestation of cattle fever ticks require systematic dipping of cattle on an infested premises every 14 d for 6–9 mo or vacating the premises for the same periods. Both options are expensive and complex. In large pastures, it is often difficult to gather 100% of the cattle for each treatment. The pasture vacation option in some cases may be ineffective because dense populations of white-tailed deer, *Odocoileus virginianus* (Zimmermann), or other wild ruminants, are capable of maintaining small populations of ticks even in the absence of cattle.

The Cattle Fever Tick Eradication Program relies solely on the use of coumaphos, an organophosphorous acaricide, in its systematic dipping of cattle. Currently, there are two major concerns associated with the reliance on this single organophosphorous acaricide: (1) whether the manufacturer will continue to support the label for this limited use, and (2) whether widespread occurrence of organophosphorous resistance in tick populations in Mexico will render the compound ineffective. There is a critical need to develop alternative acaricides and treatment methods that might have potential for use in the Cattle Fever Tick Eradication Program.

The avermectins, a class of endectocides that includes ivermectin (Campbell 1989), doramectin (Vercruysse 1993), moxidectin (Webb et al. 1991, Scholl et al. 1992, Miller et al. 1994) and eprinomectin (Shoop et al. 1996), because of their chemistry and mode of action, broad spectrum of activity and efficacy at ex-

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation by USDA for its use.

In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the Institutional Animal Use and Care Committee of the Knipling-Bushland U. S. Livestock Insects Research Laboratory, Kerrville, TX.

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tremely low dosages, represent an important class of compounds for control and management of arthropod pests of livestock. Ivermectin, the first of this class to be introduced, has been shown to control a broad variety of both endo- and ectoparasites including ticks (Drummond 1985, Lasota and Dybas 1991). Numerous studies have shown that ivermectin can control a variety of tick species (Drummond et al. 1981. Nolan et al. 1981; Lancaster et al. 1982; Miller et al. 1983, 1998; Pegram and Lemche 1985; Cramer et al. 1988; Taylor and Kenny 1990, Soll et al. 1989, 1990).

Only a few studies have investigated the potential of using ivermectin in an eradication program. Pound et al. (1996) developed technology for the delivery of ivermectin using medicated corn for the control of lone star ticks, *Amblyomma americanum* L. The medicated-bait technology was subsequently successfully used in conjunction with the Cattle Fever Tick Eradication Program to eliminate *Boophilus* spp. from large infested ranches in Webb County, TX (George 1996). Miller et al. (1999) demonstrated that injectable, bioabsorbable microspheres could be used to deliver ivermectin over an extended period (>12 wk) and thereby eliminate *Boophilus annulatus* (Say) from treated cattle and the infested pasture.

The Ivomec SR Bolus (Merck, Rahway, NJ) (Zingerman et al. 1997) was introduced as a means of sustained delivery of ivermectin to the rumen of grazing cattle. This ruminal bolus is a push-melt osmotic pump designed to deliver 11–13 mg/d of ivermectin over ≈135 d. If such a system were capable of providing a sufficiently high degree of control of *Boophilus* spp. on grazing cattle in South Texas over an extended period, it would provide a useful tool for the Cattle Fever Tick Eradication Program. The objectives of this study were to determine the efficacy and persistence of the commercial Ivomec SR Bolus and whether the use of the bolus might eliminate cattle fever ticks from an infested pasture.

Materials and Methods

This study was conducted at the Cattle Fever Tick Research Laboratory, USDA–ARS, Mission, TX, which is a USDA–APHIS Veterinary Services certified quarantine facility where research on *Boophilus* spp. ticks is conducted in support of the Cattle Fever Tick Eradication Program.

Two ≈7 ha (17 acre) pastures, consisting primarily of buffel grass (*Cenchurus ciliaius* L.), were infested with the Chimenea strain (F_7 generation) of *B. annulatus.* To accomplish equivalent infestation levels, cattle infested with all stages of the tick were moved into each pasture at selected intervals in the manner described by Miller et al. (1999). Pasture infestation was completed over an approximately 2-mo period from mid-June to mid-August with each pasture containing six infested Hereford heifers. About 3 wk before the scheduled treatment, six uninfested Hereford heifers were introduced into each pasture. As soon as there was evidence that adequate tick populations were established in each plot, the infesting cattle were removed and only the test cattle remained in each pasture. The six infested Hereford heifers (207.6 ± 16.5 kg) in the pasture designated as the treated pasture were each treated with a single Ivomec SR Bolus using a standard balling gun on 11 September 1997. The six infested Hereford heifers (206.8 ± 23.0 kg) in the pasture designated as the untreated pasture remained untreated as controls for comparative purposes.

A pretreatment tick count was taken on each animal in each pasture 2 wk before treatment and on the day of treatment to assess the parasitic tick population. The tick count procedure used throughout the study included counting all female ticks that were ≥5.5 mm long (standard engorging female) on the entire left side of each animal. In addition, to the count of these standard engorging females, the overall infestation level was assessed as low (50–100 ticks), moderate (100–300 ticks), or high (>300 ticks) for both adult and nymphal ticks present on the heifers. Counts were made weekly thereafter for >20 wk.

Before treatment (day 0) and at weekly intervals, two blood samples were collected from the jugular vein of each animal by using 13-m Vacutainers (Becton Dickinson, Franklin Lakes, NJ) until no ivermectin was detected in any animal for two consecutive bleeding times. Samples were analyzed using an high performance liquid chromatography method developed in our laboratory (Oehler and Miller 1989). The technique enables quantification of as little as 2 ppb of ivermectin in 5 ml of serum.

To determine if the treatment would not only control the ticks on the cattle, but also eliminate the free-living population in the pasture, two untreated, tick-free, Hereford heifers were introduced as sentinels into each pasture for 14 d at selected times during the study. Following the 2-wk period of exposure to infestation, the sentinel heifers were placed in individual stalls (3.3 by 3.3 m) in an open-sided barn for an additional 28 d to allow all female ticks to reach repletion and detach. Detached, engorged ticks from each individual sentinel heifer were collected and counted. Because the single bolus treatment provided less than complete control, the study was repeated with new heifers using two Ivomec SR Bolus per animal. After removal of the test heifers from the single bolus study, the pastures were reinfested according to the previously described scheme. The six Hereford heifers (214.0 ± 27.0 kg) in the treated pasture were treated with two Ivomec SR Bolus on 20 May 1998. The six Hereford heifers in the untreated pasture (227.7 ± 23.8 kg) were selected as the untreated controls. The group in the treated pasture was selected for treatment because the tick counts 2 wk before treatment indicated they had nearly twice as many ticks as those in the untreated pasture.

Data on the efficacy of the treatment, as measured by the relative number of standard engorging ticks in each pasture, were analyzed using analysis of variance (ANOVA) of repeated measurements (SAS Institute 1987). Because many observations indicated no engorging female ticks, all data were transformed to $\log_{10}$ (count + 1) for the ANOVA. Abbott's formula (Ab-

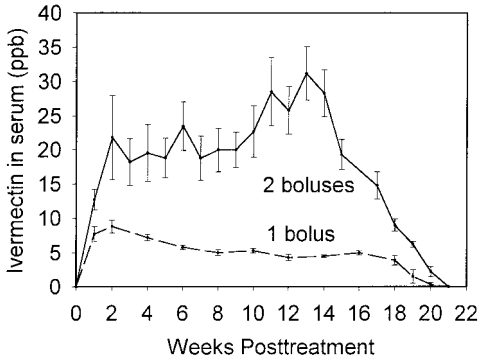

Fig. 1. Mean ± SE concentration of ivermectin in serum of six heifers as a result of the treatment with one or two Ivomec SR Boluses.

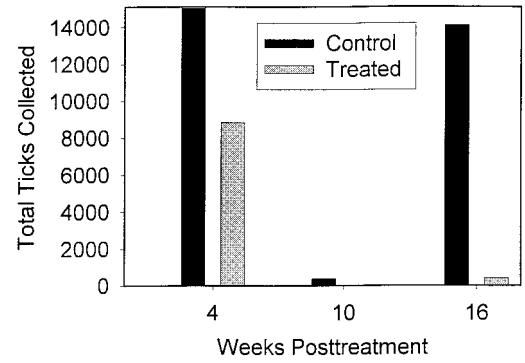

Fig. 3. Total number of engorged *Boophilus annulatus* ticks collected from two sentinel heifers placed in pastures either with heifers treated with one Ivomec SR Bolus or control heifers at indicated weeks posttreatment.

bott 1925) was applied to the geometric means of the count of standard engorging female ticks to determine overall control due to the treatment. *T*-tests were used to compare infestation levels of sentinel heifers and weight gains of cattle in the treatment pasture versus the control pasture (SAS Institute 1987).

Results and Discussion

As a result of the treatment with the single Ivomec SR Bolus, the concentration of ivermectin in the serum of the treated cattle reached a maximum of 8.8 ± 0.9 ppb at 2 wk posttreatment and steadily declined to 5.0 ± 0.3 ppb at 16 wk and 1.5 ± 1.0 ppb at 19 wk posttreatment (Fig. 1). By week 21 posttreatment, no ivermectin could be detected in the serum.

Figure 2 shows the average number of standard engorging females (≥5.5 mm long) counted on both the treated and untreated heifers after treatment with a single Ivomec SR Bolus. Cattle in the treated pasture had nearly 10 times as many standard engorging ticks than those in the untreated pasture at 2 wk before treatment (an average (±SE) 230.7 ± 29.7 verus 26.5 ± 9.2). Cattle in the treated pasture had 42.3 ± 4.5, 17.5 ± 4.2 and those in the untreated pasture had

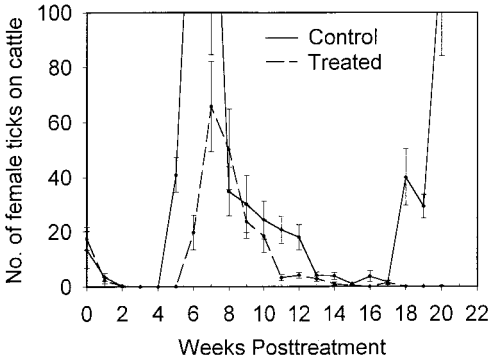

Fig. 2. Mean ± SE number of standard engorging *Boophilus annulatus* ticks (≥5. 5 mm) on six heifers treated with one Ivomec SR Bolus and on control heifers.

23.5 ± 4.6, 13.5 ± 6.7 standard engorging female ticks at 1 and 0 wk before treatment, respectively. Both pastures had high numbers of nymphs and adults 2 wk before treatment, but ticks had declined to low numbers by the week of treatment. Because of the cyclic nature of natural *Boophilus* spp. populations (Davey et al. 1994), we proceeded with the treatments.

Overall tick infestations, as well as populations of standard engorging females, on the cattle increased in both pastures between weeks 5 and 8 posttreatment. However, populations on those cattle treated with the Ivomec SR Bolus did not reach the levels of those in the control pasture. A repeated-measures ANOVA indicated a statistically significant difference in the number of standard engorging ticks on the treated cattle as compared with the untreated control cattle over the 20-wk trial ($F = 57.7$; df = 1, 10; $P < 0.001$). Abbott's formula (1925), applied to the geometric means of the standard engorging *B. annulatus* females, indicated 84.4% overall control due to the treatment of the cattle with a single Ivomec SR Bolus.

The data resulting from a 2-wk exposure of a pair of tick-free, untreated sentinel heifers in the pasture containing the treated heifers or the untreated control heifers show the potential of the technology for reducing the free-living population (Fig. 3). Although considerably fewer engorged ticks were collected from the sentinel heifers exposed in the treated pasture than in the control pasture at weeks 4, 10, and 16 posttreatment, none of the differences was significant ($P > 0.05$) because only two sentinel heifers were placed in each pasture. However, the importance of this observation is that at each exposure, free-living ticks were found in the treated pasture, indicating that the infestation was not eliminated by the treatment.

Fig. 1 also shows the ivermectin serum concentration for the cattle treated with two Ivomec SR Bolus. By the second week posttreatment, the ivermectin concentration in the serum of the treated cattle reached 21.8 ± 6.2 ppb and continued a steady rise to a maximum level of 31.2 ± 3.9 ppb at week 13. The concentration declined to 14.8 ± 2.0 ppb by week 17 and continued to decline to 2.2 ± 0.8 ppb at week 20

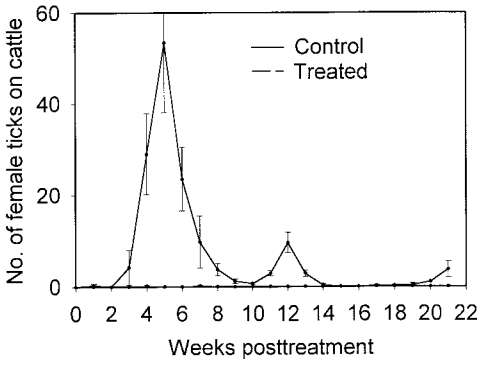

Fig. 4. Mean ± SE number of standard engorging *Boophilus annulatus* ticks (≥5.5 mm) on six heifers treated with two Ivomec SR Boluses and on control heifers.

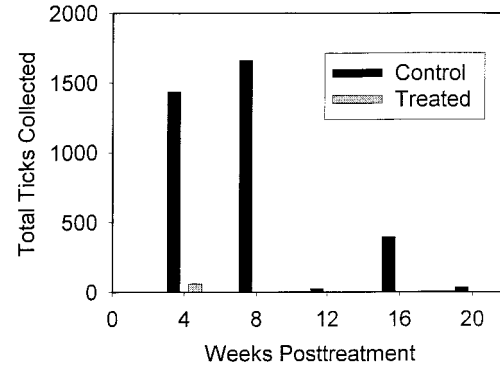

Fig. 5. Total number of engorged *Boophilus annulatus* ticks collected from two sentinel heifers placed in pastures either with heifers treated with two Ivomec SR Boluses or control heifers at indicated weeks posttreatment.

posttreatment. By week 21, ivermectin could not be detected in the serum of any treated animal. The ivermectin serum concentration profile for the two-bolus treatment trended upward from the 2-wk level until week 13, unlike the downward trend observed in the single bolus treatment.

The average number of standard engorging females counted on both the heifers treated with the two Ivomec SR Bolus and those untreated are shown in Fig. 4 for the 21-wk of the study. Two weeks before the scheduled treatment, cattle in both pastures had high numbers of nymphs and adults and cattle in the treated pasture had an average (±SE) 858.5 ± 132.0 standard engorging female ticks and those in the untreated pasture had 419.2 ± 96.3 standard engorging female ticks. Because of these heavy tick burdens and concern for the well-being of these heifers, the cattle in both pastures were treated with a 0.165% coumaphos spray at the rate of ≈2 liters/animal. Such a treatment can be expected to have a residual efficacy of <1 wk (Davey et al. 1983, Davey and George 1999). Consequently, at the time of treatment, the cattle in both the treated and the control pastures had only a low number of nymphs and adults and no standard engorging females (≥5.5 mm). The untreated control group supported a low level of nymphal and adult infestations throughout the study. Although the treated group had low levels of nymphs and adults before the week 9 posttreatment, no ticks were found on any animal thereafter. Only two standard engorging females were found on the treated heifers during the entire trial; one was found on week 4 and one on week 7 posttreatment.

Although overall tick infestations, as well as populations of standard engorging females on the cattle in untreated control group remained low throughout the study, a repeated measures ANOVA indicated a significant difference in the number of standard engorging ticks on the treated cattle as compared with the untreated control cattle over the 20-wk trial ($F = 86.2$; df = 1, 10; $P < 0.001$). The use of two Ivomec SR Bolus/heifer resulted in 99.6% control of standard engorging *B. annulatus* females for the 20-wk trial as determined by Abbott's formula (1925).

Fig. 5 shows the number of engorged ticks collected from a 2-wk exposure of a pair of tick-free, untreated sentinel heifers in the pasture containing the heifers treated with two Ivomec SR Bolus and the untreated control heifers. For each exposure period, fewer engorged ticks were collected from those sentinel heifers exposed in the treated pasture than from those exposed in the untreated pasture. However, only those sentinel heifers introduced during weeks 8 and 16 produced a statistically significantly ($P < 0.05$) different number of ticks. The low number of engorged ticks on sentinel heifers exposed in the untreated pasture during weeks 12 and 20 obscured the statistical significance of any difference in counts for those periods. However, most importantly, no engorged ticks were collected from the sentinel heifers placed in the pasture with the treated heifers after week 8 posttreatment. From this, we also conclude that the treatment of heifers with two Ivomec SR Bolus eliminated free-living ticks from the pasture.

In both the single-bolus trial and the trial with two boluses, a hand-held metal detector verified the presence of the bolus in the reticulum of each animal at the end of each trial. However, during the week 19 of the two-bolus trial, a bolus was found in the gathering pen immediately after the heifers had been worked. Although the bolus was found covered with manure, it is more likely to have been regurgitated than to have traveled through the digestive tract.

No statistical significant difference ($P > 0.05$) was found in the weight gain of the treated and the control groups for either trial. Although we have observed a greater weight gain in cattle protected from *B. annulatus* using ivermectin (Miller et al. 1999), in those trials the control cattle had higher levels of tick infestations.

Despite having treated cattle of similar body weight, the concentrations of ivermectin in serum resulting from the single Ivomec SR Bolus observed in this study are different than those previously reported by either Zingerman et al. (1997) or Alvinere et al. (1999). Zingerman et al. (1997) reported a steady-state con-

centration of 11–13 ng/m (ppb) in the plasma of bolused cattle, whereas Alvinere et al. (1999) found the level to be 20–28 ng/m. Davey et al. (2001) showed that cattle treated by oral capsule at a rate of 50 μg/kg/d had an ivermectin serum level in the range of 10–12 ppb for cattle confined to a stall. Our results with pastured heifers that showed concentrations of 5–9 ppb ivermectin in the serum are more consistent with the 6–11 ppb reported by Herd et al. (1996).

The level of tick control observed in these studies is consistent with that expected for the concentration of ivermectin in serum of the treated cattle. Drummond et al. (1981) reported that 50 μg/kg/d administered by daily oral capsule was highly effective in preventing engorgement and reproduction of adults of six species of three-host ticks and all parasitic stages of *Dermacentor albipictus* (Packard), a one-host species. Davey et al. (2001) found that daily oral capsules at a treatment level of 50 μg/kg provided >99% control of all parasitic stages of *B. microplus* (Canestrini). The Ivomec SR Bolus delivers an estimated 12 mg ivermectin/d or 60 μg/kg/d to a 200-kg heifer. However, it should be noted that for the same oral dosage, ivermectin concentrations in serum can be much reduced in cattle allowed to graze on pasture compared with cattle held in stalls (Miller and Oehler 1996).

From these studies, we conclude that a single Ivomec SR Bolus is incapable of sufficient control of *B. annulatus* to meet the rigid requirements of the Cattle Fever Tick Eradication Program in South Texas. Although two boluses per animal did eliminate the ticks from treated heifers and the pasture they were in, it is doubtful that the treatment would be sufficiently efficacious for mature cattle (>400 kg) because of the lower dose (μg ivermectin/kg body wt) for it to be useful in the program.

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